

Ion Resonance Electromagnetic Field Stimulation of Fracture Healing in Rabbits with a Fibular Osteotomy

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Summary: Rabbits with a fibular osteotomy were exposed for 28 days to magnetic fields that satisfied the ion resonance conditions for calcium or magnesium. The rabbits were exposed to whole body treatment for 1/2 hour, 3 hours, or 24 hours per day. The fibulae from the experimental and control animals were removed surgically and were subjected to force-deflection testing to establish the stiffness of the healed fracture. The fibulae from the rabbits exposed to the ion resonance magnetic fields were found to be 55-299% ($p < 0.01$) more robust than the fibulae from the control animals.

There is an extensive history of investigations of the effects of low level electrical and magnetic fields on biological systems (1,3-7,14,16,18,21,31,32,36,37). One study (8) of particular interest showed that the efflux of ions of [^{45}Ca]calcium from chick brain tissue could be enhanced 18-19% with the application of a carrier frequency modulated by a low level, sinusoidal electromagnetic signal. The effect was observed over a very narrow range of signal frequency, peaking at about 14 Hz.

Certain combined static and alternating magnetic fields that satisfy the conditions for ion resonance have been identified (25) that apparently can influence a biological endpoint in single-cell organisms and complex tissues. One test system (30), the marine diatom *Amphora coffeaeformis*, responded to the application of resonance magnetic fields, showing a significant alteration of motility patterns. Another test system (24) demonstrated that passage of ^{45}Ca into mixed human lymphocytes could be

increased more than 2-fold when the cells were exposed to resonance magnetic fields. A third study (38) involving 8-day chick embryo femora clearly demonstrated that the growth and differentiation of a complex tissue mix could be altered significantly when exposed to the resonance fields. Chick bones exposed to calcium and magnesium ion resonance magnetic fields showed accelerated growth and development, whereas those exposed to potassium ion resonance magnetic fields had inhibited growth and development. The purpose of the present project was to determine if the process that caused the enhanced growth and development in the chick femur would have a beneficial influence on bone healing in a mammalian system.

Early studies involving the use of inductively coupled pulsed electromagnetic fields were done by Bassett et al. (2). Beagles that had bilateral fibular osteotomy wore wire coils adjacent to the trauma site. The power units on the dogs generated a pulsed magnetic field through the experimental leg at a 90° angle to the fracture; the other leg served as a control. In 10 of the 13 beagles, strength was 89% greater in the experimentally stimulated leg than in the control (not significant at the 95% confidence interval). In the other three dogs, there was an accu-

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culated average of 40% greater stiffness in the control leg compared with the stimulated leg.

Brighton et al. (11) utilized capacitively coupled electric fields on rabbits with a fibular osteotomy. This system also elicited a positive response in healing fractures. The experimentally exposed bones developed 93% greater stiffness than the control bones.

We hypothesized that the ion resonance electromagnetic stimulation that caused these changes in cell membrane ion flux and *in vitro* bone tissue might have a similar influence on the cells comprising callus at a fracture site in a rabbit fibula and favorably influence the characteristics of the callus, as evidenced by greater stiffness and histologic maturity.

METHODS

Operative Procedures

The study involved 36 experimental and 18 control New Zealand White rabbits of randomly mixed sex. The hindlimbs were shaved from hip to ankle, and the rabbits were anesthetized with ketamine (45 mg/kg of body weight) and xylazine (10 mg/kg of body weight). Under sterile conditions, a 2.5 cm incision was made, beginning 1.5 cm distal to the knee joint, on the lateral surface of each leg at the junction of the anterior and lateral muscle compartments. The anterior compartment muscle was divided from the lateral compartment muscle by anterior retraction of the anterior compartment and posterior retraction

of the lateral compartment. This division was deepened until approximately 1.5 cm of the fibula was exposed. A 1.0 cm incision was made in the fibular periosteum beginning 5-8 mm above the tibiofibular junction. Then, a rongeur was used to remove a 1.0 cm section of the fibula from the left leg, with the periosteum left in place. No bone was removed from the right leg, and the periosteum was allowed to close. All experimental and control animals had resection on the left fibula and a sham operation on the right. In the experimental animals, the left leg served as the experimental side and the right leg served as the control for baseline differences in bone strength among animals. The superficial and deep layers of the wound were closed with 3-0 Dexon (polyglycolic acid) soluble sutures (Ethicon, Somerville, NJ, U.S.A.). After the rabbits recovered from anesthesia, a postoperative analgesic (buprenorphine, 0.3 mg) was administered.

The animals were assigned randomly to cages with or without activated electromagnetic coils; the cages with the activated coils were 3 m from the control cages. During the experimental period, the animals were unrestrained and free to move about in their cages and were supplied with 100 g of feed daily. The temperature in the room was controlled to $23 \pm 1^\circ\text{C}$, the light cycle was a 12/12-hour light/dark schedule, and the humidity was maintained at approximately 50%.

In the experimental protocol, 18 control animals

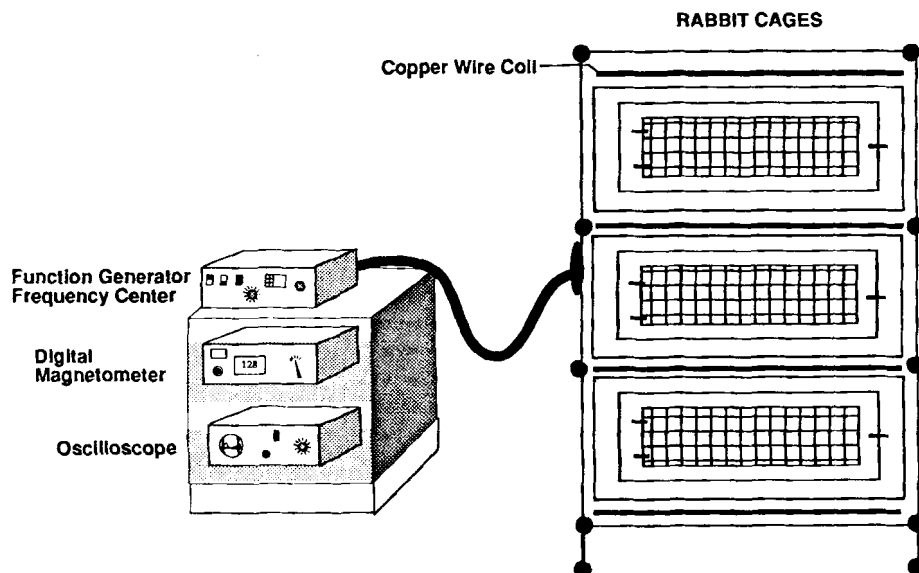


FIG. 1. Schematic diagram of the rabbit cages and equipment used to generate and calibrate the resonance magnetic fields. The function generator supplied the alternating current component of the magnetic field. The frequency counter (magnetometer and oscilloscope) was used to measure and calibrate the system.

were not exposed to magnetic fields. The 36 experimental animals were divided into 12 groups of three animals each. The magnetic field induced in the cages with activated coils had a frequency of 16 Hz and an intensity of 209 mG (2.09×10^{-5} T) for calcium ion resonance and 127 mG (1.27×10^{-5} T) for magnesium ion resonance. The magnetic fields were activated for 1/2 hour, 3 hours, or 24 hours per day and were adjusted to influence calcium ions for three animals and magnesium ions for three (for a total of six experimental runs). The three remaining animals in each group served as a simultaneous control.

Experimental Stimulation

Experimental resonance magnetic fields were generated and calibrated in the following manner. Amplitudes of the alternating and static magnetic fields were measured with a digital magnetometer (model DM 2220-S5; Schonstedt, Reston, VA, U.S.A.). To read the amplitude of the alternating current, the analog output from the magnetometer was fed to a calibrated oscilloscope (204 A; Tektronix, Beaverton, OR, U.S.A.) (Fig. 1). The coils were set to provide vertical magnetic fields, and the vertical component of the geomagnetic field was measured at the center of the rabbit cages. This value was used as B_s in Eq. 1, which establishes a physical relationship describing resonance frequency:

$$fA = ([q/m])Bs/2\pi \quad (1)$$

where f is the resonance frequency (Hz), q is the charge (C), m is the mass (kg), and B is the magnetic field intensity (T).

The appropriate ratio of charge to mass depended on which ionic stimulation, calcium or magnesium, was to be tested. The use of the vertical geomagnetic field as the B field provided a stable, uniform direct current magnetic field. All fields were monitored daily, and the frequency was adjusted appropriately, if necessary, to maintain resonant conditions. The peak-to-peak amplitude of the time-varying magnetic field was set to 30.0 μ T.

Sets of four-square copper magnet wire coils (400 turns of No. 24 American Wire Gauge magnet wire), properly spaced to establish Helmholtz-aiding conditions, surrounded the top and bottom of stacks of three cages 60 cm long $\times$ 60 cm wide $\times$ 40 cm deep. A function generator (FG-2 Circuitmate; Beckman Instruments, Fullerton, CA, U.S.A.) was connected to the coils, and the resonance frequency (fA , Eq. 1) was set with a frequency counter (Circuitmate UC-10; Beckman Instruments). The function generator

supplied the appropriate sine wave magnetic field along the axis of the coils.

Collection and Analysis of Data

All measurements and tests on the animals were conducted in a blinded manner. Twenty-eight days after surgery, the rabbits were weighed and then killed, and each hindlimb was disarticulated. Radiographs were obtained with the specimen positioned directly on the film cassette, with the anterior border of the tibia contacting the cassette. Thus, the fibular radiograph used a posteroanterior projection. The fibula was removed from the specimen, cleaned of adherent tissues, and subjected to a cantilever bending test to determine the force required to produce 1.0 mm of deflection. This parameter was measured within 2 minutes after dissection of the fibula, thereby minimizing drying. To perform the stiffness tests, the distal end of the fibula was clamped in a horizontal micromanipulator and the proximal end was moved against a force-displacement transducer (FT-10; Grass Instruments, Quincy, MA, U.S.A.). The transducer came in contact with the bone 1.0 cm above the clamp, and the clamped end of the bone was moved toward the transducer with a uniform displacement rate of 1.0 mm/sec in the anteroposterior plane of the fibula (Fig. 2). The resultant force

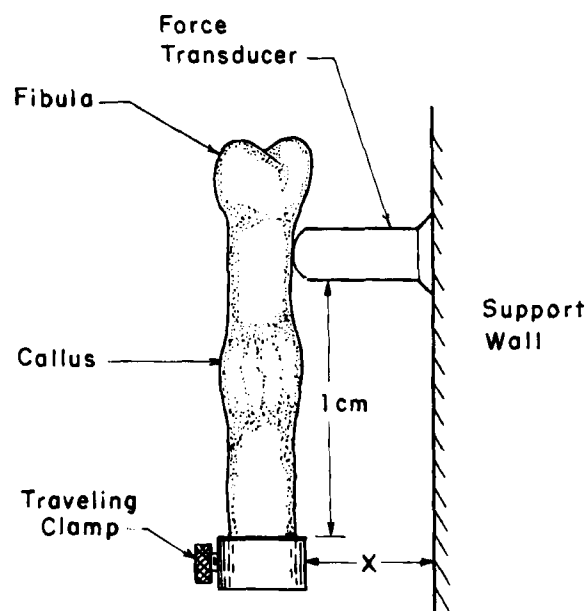


FIG. 2. Schematic diagram of the apparatus to measure bone strength. X is the distance between the force transducer support and the bone clamp. After contact of the fibula with the force transducer, the clamp is moved an additional 1 mm (X is reduced by 10.0 mm), and the resultant force is recorded.

TABLE 1. *The influence of ion resonance magnetic fields on bone properties and total body weight*

	Body weight (kg)	Diameter (mm)		Force-deflection ratio (ostectomy/sham operation)
		Callus	Fabella	
Control	2.59 ± 0.14	2.15 ± 1.18	2.62 ± 0.47	0.67 ± 0.37
Calcium ions				
1/2 hour	2.64 ± 0.15	3.63 ± 0.56 ^a	2.98 ± 0.45	1.84 ± 1.31 ^a
3 hours	2.48 ± 0.18	3.66 ± 0.58 ^a	3.50 ± 0.45 ^b	1.80 ± 1.33 ^a
24 hours	2.40 ± 0.12 ^a	4.19 ± 0.67 ^b	3.41 ± 0.25 ^b	2.67 ± 1.76 ^b
Magnesium ions				
1/2 hour	2.74 ± 0.12 ^a	4.18 ± 0.97 ^b	4.02 ± 0.42 ^c	1.04 ± 0.73
3 hours	2.57 ± 0.09	3.09 ± 0.43	3.37 ± 0.19 ^b	1.85 ± 0.98 ^b
24 hours	2.72 ± 0.39	3.60 ± 0.79 ^a	3.31 ± 0.48 ^a	1.59 ± 0.78 ^b

Values for body weight and diameter are given as the mean ± SD, and values for force-deflection ratio are given as the mean ± SEM. There were 18 control animals, but only 15 specimens were available for measurement of callus and of force-deflection ratio. There were six animals in each experimental group, but only five specimens were available for measurement of callus and of force-deflection ratio for the groups exposed for 3 hours and 24 hours to calcium or magnesium ions.

^aSignificantly different from control ($p < 0.05$, Student *t* test).

^bSignificantly different from control ($p < 0.01$, Student *t* test).

^cSignificantly different from control ($p < 0.001$, Student *t* test).

was recorded in millivolts. The data were collected with use of a dynagraph amplifier (R612; Beckman Instruments) fed into a personal computer-based data acquisition system (Computerscope; RC Electronics, Santa Barbara, CA, U.S.A.) that measured the peak amplitude of each of four repeated load-deflection responses on each bone. Ratios were calculated for force-deflection values in the left limb (ostectomy) compared with the right limb (sham operation).

After the fibulae had been tested, they were preserved in 10% Millonig's neutral buffered formalin and prepared for histological examination. Soft-tissue samples were removed from each animal, and histological preparations were made from the lung, gonads, kidney, liver, spleen, and heart. The fibula was decalcified and embedded, and longitudinal sections, 5.0 µm thick, were stained with hematoxylin and eosin. Similar 5.0 µm sections were prepared for all soft-tissue samples. Additionally, measurements of the right-left diameter of the middle of the fibular callus and the fabella were made on the radiographs with use of digital calipers. The fabella is a small sesamoid bone in the lateral head of the gastrocnemius muscle. It was measured to assess influences on generalized bone growth.

There were seven nonunions in the study: three in the control group and four among the experimental groups. No experimental group had more than one nonunion. Since these seven fibulae had no callus, the force-deflection ratio (ostectomy/sham operation) was essentially zero, and therefore they were excluded from statistical analysis of callus stiffness

and from the morphometric parametric data. We believed that if no callus was present, it was unreasonable to include such data when asking questions concerning the characteristics of calluses.

In the statistical analysis of the parametric data, calculated means were used except for the force-deflection values, for which geometric means were used due to the comparison of ratios rather than absolute values.

The data also were analyzed by Student's *t* test. All values were presented with SDs except for the force-deflection ratios, which were reported with SEMs. A comparison of control and experimental parameters were reported for each experimental category as a percentage increase or decrease from control values, as expressed by *P* in

$$P = ([\text{experimental} - \text{control}]/\text{control}) \times 100 \quad (2)$$

Histologic sections were studied to determine (a) if the soft tissues were normal in appearance for the tissue type and (b) if any morphologic abnormalities were present. Slides of bone tissue (callus) were examined with a microscope (CH-2; Olympus, Lake Success, NY, U.S.A.) fitted with a 36-intersection counting grid ocular. To estimate the areas occupied by intertrabecular space, trabecular bone, and fibrocartilage, grid intersections were counted at $\times 100$ and converted to a percentage. Three contiguous fields were counted across the center of the ostectomy site as a transit, and the values were averaged to represent that particular bone. The amount of lacunar area (percentage of trabecular bone occupied by osteocytic lacunae) within the bone was estimated: five fields were selected at random from the central

ostectomy trabeculae lying along the previously counted transit line and were counted at $\times 200$. The five values also were averaged and recorded as representative of that particular bone. One control ostectomy site was lost during processing; therefore, there were 14 control tissues in the histology study and 15 in the gross studies.

RESULTS

There were significant differences in the force-deflection ratios between experimental and control animals (Table 1). In animals exposed to calcium ion magnetic resonance for 1/2 hour per day, there was a 175% increase in stiffness compared with control animals ($p < 0.05$). Fibulae from rabbits exposed to calcium ion magnetic resonance for 3 hours per day were 169% stiffer than fibulae from control animals ($p < 0.05$). The greatest increase (299%) in stiffness was seen in rabbits exposed to calcium ion magnetic resonance for 24 hours per day ($p < 0.01$).

In animals exposed to magnesium ion magnetic resonance, there was a 55% increase in relative stiffness (not significant at the 95% confidence level) after exposure for 1/2 hour per day, a 175% increase in relative stiffness ($p < 0.05$) after exposure for 3 hours per day, and a 137% increase in relative stiffness ($p < 0.01$) after exposure for 24 hours per day.

The diameter of the callus increased progressively as the exposure time to magnetic fields selected for calcium ions increased. There was a 69% increase ($p < 0.05$) with exposure for 1/2 hour per day, a 70% increase ($p < 0.05$) with 3 hours per day, and a 95% increase ($p < 0.01$) with 24 hours per day. When magnetic fields selected for magnesium ions were employed for 1/2 hour per day, the diameter of the callus was 94% more than that of the controls ($p < 0.01$). Magnesium ion magnetic resonance produced a 44% increase (not significant) after exposure for 3 hours per day and a 67% increase ($p < 0.05$) after exposure for 24 hours per day. The radiographs in Fig. 3 illustrate the difference in the diameter of the fracture callus between an animal exposed to magnetic fields selected for magnesium ions for 1/2 hour per day (4.91 mm) and a control animal (2.45 mm).

The changes in the diameter of the fabella (sesamoid bone) were not significant in animals that had been exposed to magnetic fields selected for calcium ions for 1/2 hour per day (an increase of 14%), whereas in animals that had been exposed to fields for 3 and 24 hours per day, the diameter increased 34% ($p < 0.01$) and 31% ($p < 0.01$), respectively.

There was a 54% ($p < 0.001$) increase in the diameter of the fabella in animals exposed to magnesium ion magnetic resonance for 1/2 hour per day. When exposure to these fields was increased to 3 and 24 hours per day, the increases were 29% ($p < 0.01$) and 26% ($p < 0.05$), respectively.

The weight of each animal was recorded at the end of the study to determine if the weights of the experimental and control animals differed significantly. All changes in weight of the experimental animals were within 10% of the weight of the control animals. The variations that were statistically significant ($p < 0.05$) occurred in the group exposed to magnetic fields selected for calcium ions for 24 hours per day and in the group exposed to fields selected for magnesium ions for 1/2 hour per day. At autopsy, the change in weight appeared to be relative to the amount of intra-abdominal fat present.

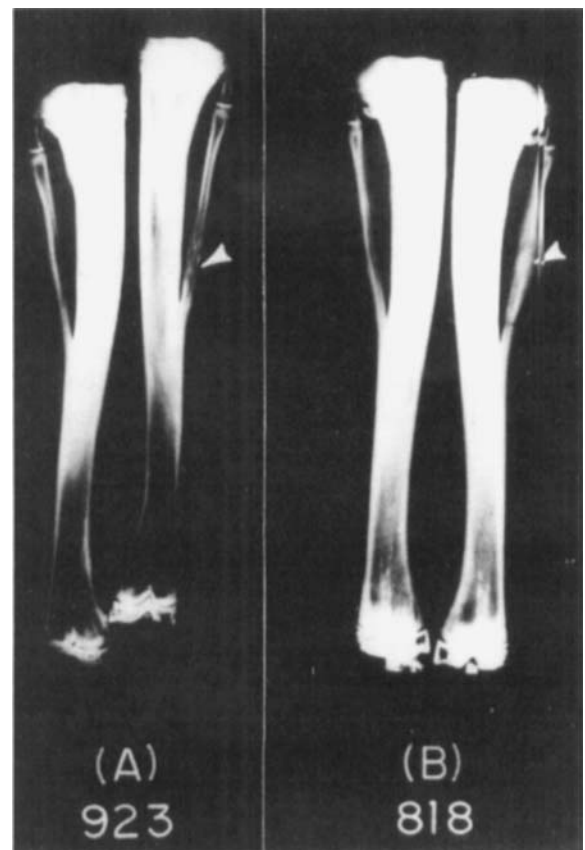


FIG. 3. Radiographs demonstrating the increase in the diameter of the callus due to exposure to ion resonance magnetic fields. The arrows indicate the site of the fibular ostectomy and subsequent formation of callus. The diameter of the callus was 2.45 mm in a control animal (A) and 4.91 mm in an animal exposed to magnesium ion resonance magnetic fields for 1/2 hour per day (B).

TABLE 2. Results of histologic assessment of mid-callus

	Percentage of osteotomy site			
	Intertrabecular space	Intertrabecular bone	Lacunar area	Fibrocartilage
Control (n = 14)	38 ± 14	39 ± 19	11 ± 2	22 ± 20
Calcium ions				
1/2 hour (n = 6)	21 ± 7 ^a	61 ± 19 ^a	13 ± 2	15 ± 12
3 hours (n = 5)	25 ± 6 ^a	59 ± 9 ^b	11 ± 0	13 ± 6
24 hours (n = 5)	15 ± 7 ^c	76 ± 16 ^c	13 ± 2	8 ± 5
Magnesium ions				
1/2 hour (n = 6)	21 ± 5 ^b	68 ± 8 ^c	12 ± 2	11 ± 6
3 hours (n = 5)	24 ± 9 ^a	57 ± 16 ^a	12 ± 3	15 ± 9
24 hours (n = 5)	18 ± 6 ^b	65 ± 15 ^b	12 ± 1	9 ± 7

Values are given as the mean ± SD. The number of specimens reflects the omission of seven nonunions and one control specimen that was lost in the tissue processing laboratory.

^aSignificantly different from control ($p < 0.05$, Student *t* test).

^bSignificantly different from control ($p < 0.02$, Student *t* test).

^cSignificantly different from control ($p < 0.001$, Student *t* test).

The results of the histological examination of bone are presented in Table 2. All stimulated (experimental) osteotomy sites had significantly less intertrabecular space and significantly more bone compared with the controls. The spacing of osteocyte lacunae in the newly formed trabeculae essentially was unaffected by resonance stimulation. Analysis of the sections revealed 32-64% less cartilage in the experimental animals than in the controls. However, the variability of the results was so great that no experimental values were significantly different from control values at the $p < 0.05$ standard. In every case, however, the amount of cartilage present was less than in the controls; the maximum difference occurred after stimulation with calcium ion magnetic resonance for 24 hours per day. Thus, there was a trend toward more mature callus in the experimental animals, and this was significant in a chi-square test ($p < 0.01$) and in a sign test ($p < 0.01$).

Histologic analysis of the tissue sections revealed no abnormal cell architecture or morphology in the organs harvested from the rabbits (control or experimental) in this study.

DISCUSSION

The results of these experiments suggest that complex physiological processes can be influenced by ion-specific resonance magnetic fields in an *in vivo* mammalian system. Previous studies (30,38) have demonstrated that single-cell organisms and an *in vitro* cell system could be influenced by such magnetic fields. There appears to be a correlation between the results in the previous *in vitro* study on chick bone (38) and the current *in vivo* study on rab-

bit bone. Chick femoral rudiments exposed to calcium or magnesium resonance magnetic fields demonstrated increases in both generalized growth and maturation, which correlates well with the results on rabbit fibulae in this study.

There may be a common mode of influence by the resonance magnetic fields on the biological systems represented. A previous study (24) showed that intracellular concentrations of radiolabeled calcium ions were increased compared with controls when cells were exposed to ion resonance magnetic fields. This process may have been occurring in the osseous tissue culture (38) and the fracture sites on rabbit fibulae in our study, although experimental evidence is lacking.

There are several proposed mechanisms for the observed effects of weak magnetic fields on biological systems. Rooze and Hinsenkamp (35) hypothesized that the modulating effect of pulsed electromagnetic fields is based on the field directly advancing ionic adsorption at the cell membrane surface, as well as on induction of structural changes in membrane proteins. Liboff (23) postulated more specifically that the site of action was the membrane ion channel, which may have a similar internal structure in many different species (12). Liboff (23) suggested that cyclotron resonance may be occurring for ions in the membrane channels during a helical transit. The structure of membrane channels may indeed induce a helical ion path (9,12,15,17,19,20,33,34,39). Transit through this pathway may be enhanced by resonance conditions, as it is known that Lorenz forces present in a resonance tuned field in free space cause a charged ion to travel in a helical manner (28,29). In this model,

ions in a membrane channel experience resonance conditions that depend on the ratios of charge to mass (Eq. 1, q/m , in coulombs/kilograms) of the unhydrated ion and the intensity of the local magnetic field (B_s , in Teslas).

Alternatively, Chiabrera et al. (13) hypothesized that resonance magnetic fields may influence the binding process of a ligand to its receptor site on the glycocalyx of a cell membrane. This process may be related to an interaction where a charged ion sheds its water of hydration at the cell membrane surface and subsequently has rare interactions with water molecules.

Lednev (22) hypothesized a physical mechanism for resonance interactions of weak magnetic fields with biological systems. He suggested that an ion incorporated into a binding protein is a model of a charged oscillator. A change in the interaction of the ion with its surrounding ligands may be produced by a shift in ion transitions between vibrational energy levels caused by the application of static and alternating magnetic fields, a phenomenon known as parametric resonance. The greatest effect is seen when the frequency of the alternating field is equal to the cyclotron frequency of the ion.

Brighton et al. (10) postulated that oscillating electric fields may have a direct effect on bone. The oscillating electric field may act as a primary messenger that activates a second messenger system (i.e., the cyclic adenosine monophosphate [cAMP] system in skeletal cells). In turn, the second messenger stimulates the enzymatic processes directing osteogenesis.

Luben and Cain (26) suggested that pulsed electromagnetic fields may interfere with the parathyroid hormone-stimulated calcium loss from skeletal tissue. Pulsed electromagnetic fields may affect the reduction of a molecule constituting a part of the membrane receptor for parathyroid hormone. The change in the membrane receptor blocks the receptor's interaction with adenylate cyclase, thus preventing the stimulatory activity of the hormone (26,27).

The data presented in this study demonstrate that, in animals exposed to calcium and magnesium resonance magnetic fields after fibular osteotomy, the stiffness of the fracture callus was increased significantly (as much as 299%, $p < 0.01$) compared with controls. It should be noted that a subperiosteal osseous defect was created in these animals by removal of 1 cm of fibula and not by an apposed transverse fracture. Future investigative efforts will focus on further elucidation of the mechanism of action of

ion cyclotron resonance electromagnetic fields and determination of their influence in other biological systems.

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